DESCRIPTION

HOMEOBOX GENE ENCODING A PROTEIN INVOLVED IN DIFFERENTIATION

Technical Field

The present invention relates to gene encoding a protein that is involved in differentiation and that has a homeodomain-like sequence. More specifically, the present invention relates to genes encoding a protein that has an ability of inducing adventitious shoots and branching and that has a homeodomain-like sequence, and uses thereof.

Background Art

Plants generally have totipotency and, for example, can regenerate individual plants through the regeneration of adventitious shoots or adventitious embryos from undifferentiated tissues derived from somatic cells. This ability is used for, for example, the production of young plants by cultured shoot. In addition, the technique of regenerating transformed plants via the regeneration of adventitious shoots or adventitious embryos after the introduction of genes into plant somatic cell tissues or cultured plant cells has become an indispensable technology in the field of plant biotechnology in recent years. It is generally thought that the regeneration of adventitious roots or adventitious shoots from callus, which is an undifferentiated cells or plant tissues originated from leaves, stems, and the like is regulated by the interaction of plant hormones such as auxins and cytokinins.

For plant morphogenesis, it has also been reported that a series of genes including homeobox are involved in addition to plant hormones. Homeobox genes was found as a well-conserved 183 bp DNA sequence occurring in common

in certain genes that regulate the development of Drosophila. The 61 amino acid sequence translated from this region is called homeodomain, which takes a helix-turn-helix structure comprising three α helixes and which recognizes a specific nucleotide sequence thereby to bind DNA.

Animal homeobox genes have been elucidated to be transcription factors that control development processes, whereas for plants the KNOTTED1 (KN1) gene isolated from corn in 1991 is the first homeobox gene in higher plants (Vollbrecht et al., Nature 350: 241-243, 1991). Although veins of corn leaves are parallel ones, Knotted1 mutation results in disturbances in veins and drives formation of knot-like processes along veins, after which it was named Knotted.

On the other hand, using synthetic DNA corresponding to specifically highly conserved amino acid sequences in the homeobox that had been found in many animals, genomic DNA of a dicotyledon *Arabidopsis thaliana* was searched with a result that several homeobox genes were reported (Ruberti et al., EMBO J. 10: 1787-1791, 1991).

Homeobox genes of higher plants reported so far have been roughly grouped into five types based on the similarity of amino acid composition in the homeodomain and the structure of regions other than the homeobox domain (Tasaka, Tanpakushitu Kakusan Koso (Proteins, Nucleic Acids, Enzymes) 40(8): 1033-1042, 1995). The first type is represented by the KN1 gene of corn, the second type has the homeobox approximately in the center of a protein, of which a C-terminal end is flanked by a regularly repeated structure of leucine moieties (leucine zipper) that are involved in dimer formation of the protein. The third type has the homeodomain near the C-terminal end of a protein, and a finger structure of the metal-bound type at the N-terminal end. The fourth type, in addition to having a structure common to the third type, has repeated structures of several amino acid

sequences. The fifth type has the homeobox in the N-terminal end of a protein and no other well-known characteristic structures have been found therein.

The overall homology of amino acid sequences between the different types is 32 to 58% in the homeodomain. However, as can be estimated from a report that the third helix in the homeodomain enters into the main groove of the target double stranded DNA to control transcription when a protein containing an animal homeodomain binds to DNA, this third helix has the highest homology irrespective of the type even in the gene products of plant homeoboxes. The region is thought to be essential for a homeodomain protein to bind to DNA as a transcription factor. Recently, a homeobox gene WUSCHEL was reported that does not belong to any of these five groups (Cell, 95: 805-815, 1998). Although the mutants defective in the function of the WUSCHEL gene cannot drive normal growth of apical meristem of the stem, there are no experimental reports on overexpression of the WUSCHEL gene, and it is unknown what changes may occur when the WUSCHEL gene expression is artificially enhanced.

The homeobox genes of plants have been suggested to be possibly involved in the control of organogenesis or development processes, infection protection, and regulation of material transport in the plants, details of which are not known, however. Protein having a homeobox is generally thought to serve as transcription factor, but even the target gene whose transcription is regulated by each homeodomain protein has not been elucidated. Furthermore, although the overexpression of the KN1 type among the homeobox genes causes extremely abnormal morphology, it is not known whether adventitious shoots are formed on the callus.

From the standpoint of agricultural application, a gene having a high ability of inducing adventitious shoots and branching on a cultured tissue such as callus

in the tissue culture system would be considered to be useful, but there are no such genes.

Disclosure of the Invention

Thus, it is an object of the present invention to provide a gene encoding a protein that is involved in differentiation and that has a homeodomain-like sequence, specifically a protein that has an ability of inducing adventitious shoots and branching, a protein encoded thereby, and uses thereof.

The inventors of the present invention conducted activation tagging using *Arabidopsis thaliana* and have obtained a gene encoding a protein that has an ability of inducing adventitious shoots and branching. The activation tagging as used herein means a method of inserting enhancer sequences at random into a plant genome to isolate mutants in which the transcription of genes downstream of the inserted enhancer has been activated.

Thus, the present invention provides a gene encoding a protein that is involved in differentiation and that has a homeodomain-like sequence. More specifically, the present invention provides a gene encoding a protein that has an ability of inducing adventitious shoots and branching, and that has a homeodomain-like sequence.

More specifically, the present invention provides a gene encoding a protein that has the amino acid sequence as set forth in SEQ ID NO: 2, that is involved in differentiation, and that has a homeodomain-like sequence. The present invention further provides a gene encoding a protein that has an amino acid sequence modified by the addition or deletion of one or a plurality of amino acids and/or replacement with other amino acids in the amino acid sequence as set forth in SEQ ID NO: 2, that is involved in differentiation, and that has a homeodomain-like sequence. The present invention further provides a gene that hybridizes to the

nucleic acid as set forth in SEQ ID NO: 1, specifically its DNA or a portion thereof, and that encodes a protein that is involved in differentiation and that has a homeodomain-like sequence.

The present invention further provides a gene encoding a protein that has the amino acid sequence as set forth in SEQ ID NO: 4, that is involved in differentiation, and that has a homeodomain-like sequence. The present invention further provides a gene encoding a protein that has an amino acid sequence modified by the addition or deletion of one or a plurality of amino acids and/or replacement with other amino acids in the amino acid sequence as set forth in SEQ ID NO: 4, that is involved in differentiation, and that has a homeodomain-like sequence. The present invention further provides a gene that hybridizes to the nucleic acid as set forth in SEQ ID NO: 3, specifically its DNA or a portion thereof, and that encodes a protein that is involved in differentiation, and that has a homeodomain-like sequence.

As used herein, a protein that is involved in differentiation and that has a homeodomain-like sequence is a protein that is involved in the process in which cells differentiate into morphologically and/or functionally different cells such as differentiation into adventitious shoots, branches, leaves, flowers or the like, and that has a homeodomain-like sequence functioning as a DNA-binding domain, and specifically a protein that induces the formation of adventitious shoots, a protein that induces branching, and the like.

The present invention also provides vectors comprising the gene.

The present invention further provides hosts transformed with the vector. The hosts may be plant cells or plants.

The present invention also provides a method for producing a protein that is involved in differentiation

and that has a homeodomain-like sequence by culturing and/or cultivating the above host.

The present invention also provides a method for inducing differentiation of plants or plant cells, said method comprising introducing the above gene into plants or plant cells and driving the expression of said gene.

The present invention also provides a method for inducing the formation of adventitious shoots of plants or plant cells, said method comprising introducing the above gene into plants or plant cells and driving the expression of said gene.

The present invention also provides a method for inducing branch formation of plants said method comprising introducing the above gene into plants and driving the expression of said gene.

Embodiment for Carrying Out the Invention

The inventors of the present invention investigated the possibility of identifying a gene involved in differentiation that leads to the induction of adventitious shoot formation on a callus when overexpressed by activation tagging. Thus, *Arabidopsis thaliana*-transformed calluses into which an activation tagging vector pPCVICEn4HPT had been introduced via *Agrobacterium* were screened on a cytokinin-free medium to isolate a transformant that formed adventitious shoots even in the absence of cytokinin (an adventitious shoot is not usually formed in the absence of cytokinin). Among them, a transformant designated "many shoot" (msh) formed adventitious shoots in the absence of cytokinin.

When the MSH gene that caused the phenotype of the msh mutant and the corresponding MSH cDNA were isolated and analyzed, it was found that the protein encoded by the MSH gene has an amino acid sequence having a significant homology with homeodomain, and, among others, the third α helix domain of the homeodomain conserved in a series of homeodomain proteins had a high homology. In

addition, when the coding region of MSH cDNA was introduced into an *Arabidopsis thaliana* callus and was overexpressed, as can be indicated from the phenotype of the msh mutant, the transformed callus formed an adventitious shoot in the presence or absence of cytokinin in the medium. It was also found that in *Arabidopsis thaliana* transformants in which MSH cDNA was overexpressed, branching was more frequent than in the wild type *Arabidopsis thaliana*, and adventitious shoots were occasionally formed on the leaves.

The foregoing revealed that the MSH gene is involved in differentiation and encodes a protein having a homeodomain-like sequence, which indicated the possibility that the overexpression of this might result in enhanced ability of forming adventitious shoots and branching.

As the gene of the present invention, there can be mentioned one that encodes the amino acid sequence as set forth in SEQ ID NO: 2 or 4. However, it is known that a protein that has an amino acid sequence modified by the addition or deletion of one or a plurality of amino acids and/or replacement with other amino acids retain similar effects to the native protein. Thus, the present invention encompasses a protein that has an amino acid sequence modified by the addition or deletion of one or a plurality of amino acids and/or replacement with other amino acids in the amino acid sequence as set forth in SEQ ID NO: 2 or 4, and a gene encoding said protein.

As used herein, the degree of modification is one that is possible by means known prior to the filing of the present application such as site-directed mutagenesis and PCR. The number of amino acids targeted for modification while maintaining the ability of inducing adventitious shoots and branching is for example 50 or less, preferably 25 or less, for example 10 or less.

The present invention also provides a gene that hybridizes to the nucleic acid as set forth in SEQ ID NO:

1 or 3, specifically its DNA or a portion thereof under a stringent condition, and that encodes a protein that is involved in differentiation and that has a homeodomain-like sequence. The stringent condition as used herein means a condition in which hybridization occurs, for example, 5 × SSC and 50°C. A suitable hybridization temperature may be selected as appropriate since it varies with the nucleotide sequence or the length of the nucleotide sequence.

The above portion of a nucleic acid is a portion that encodes a sequence comprising at least several contiguous amino acids, and preferably a portion that encodes a sequence comprising at least several contiguous amino acids in the homeodomain. More preferably, it means a portion or a fragment that contains a part or all of the homeodomain sequence in the sequence as set forth in SEQ ID NO: 1 or 3, and that has a length of 25% or greater, for example 50% or greater, and more preferably 75% or greater of the entire sequence as set forth in SEQ ID NO: 1 or 3.

As a source of the gene as a target for the above hybridization, there can be used a cDNA library, a genomic DNA library, etc. prepared from plants, microorganisms etc., and as the plant, there can be mentioned *Arabidopsis thaliana*, petunia, snapdragons, rice, corn, tabacco, poplar, and the like.

The nucleotide sequence of the thus obtained gene encoding a protein that is involved in differentiation and that has a homeodomain-like sequence has a homology of 50% or greater, 60% or greater, preferably 70% or greater or 80% or greater, for example 90% or greater with the nucleotide sequence as set forth in SEQ ID NO: 1 or 3.

The gene of the present invention encoding a protein having the amino acid sequence as set forth in SEQ ID NO: 2 or 4 may be obtained from *Arabidopsis thaliana* as cDNA or genomic DNA.

As specifically shown in the examples, genes having the native nucleotide sequence can be obtained by, for example, screening cDNA libraries. DNA encoding a protein having a modified amino acid sequence can also be synthesized based on the DNA having the native nucleotide sequence by conventionally used site-directed mutagenesis or a PCR method. For example, a DNA fragment to be modified may be obtained by treating the native cDNA or genomic DNA with restriction enzymes, and using this as a template, site-directed mutagenesis or a PCR method is carried out using a primer into which the desired mutation has been introduced so as to obtain a DNA fragment into which the desired modification has been introduced. Then the mutation-introduced DNA fragment may be linked to a DNA fragment encoding another part of the protein of interest.

Alternatively, in order to obtain a DNA encoding a protein comprising a shortened amino acid sequence, a DNA encoding an amino acid sequence longer than the amino acid sequence of interest, for example a full-length amino acid sequence, is cleaved by a desired restriction enzyme, and when the resulting DNA fragment was found not to encode the entire amino acid sequence of interest, a DNA fragment comprising the lacking sequence is synthesized and ligated thereto.

prokaryotic or eukaryotic organism. As a prokaryotic organism, there can be used such a common host as a microorganism belonging to the genus *Escherichia* such as *Escherichia coli*, a microorganism belonging to the genus *Bacillus* such as *Bacillus subtilis*, and the like.

As an eukaryotic host, there can be used a lower eukaryotic organism, for example an eukaryotic microorganism, for example a fungus, yeast or a mold. As yeast, there can be mentioned a microorganism belonging to the genus *Saccharomyces* such as *Saccharomyces cerevisiae*, and as a mold, there can be mentioned a microorganism belonging to the genus *Aspergillus* such as *Aspergillus oryzae* and *Aspergillus niger*, and a microorganism belonging to the genus *Penicillium*. Furthermore, animal cells or plant cells can be used: as animal cells, there can be used cell lines derived from mouse, hamster, monkey, human and the like, specifically COS cells, Vero cells, CHO cells, L cells, C127 cells, BALB/c3T3 cells, Sp-2/0 cells, and the like. As plant cells, there can be used cultured cells from tabacco, genus *Populus*, genus *Eucalyptus*, genus *Acacia*, and the like.

Insect cells such as silkworm cells or adult silkworms per se can also be used as hosts. Specifically, insect cells such as cells of *Spodoptera frugiperda*, cells of *Bombyx mori*, etc. may be used.

As vectors, there can be used plasmid, phage, phagemid, virus (baculovirus (insect cell expression

like; as promoters for yeasts, there can be used glyceraldehyde-3-phosphate dehydrogenase promoter, PH05 promoter, adhI promoter, pqk promoter and the like; and as mold promoters, there can be used amylase promoter, trpC promoter, and the like.

As promoters for insects, there can be mentioned the baculovirus polyhedrin promoter etc.; as animal cells, there can be mentioned the early and late promoter of Simian Virus 40, CVM promoter, HSV-TK promoter or SRα promoter, and the like.

Furthermore, as promoters for plants, there can be mentioned CaMV35S promoter, nopaline synthase promoter; as inducible promoters, there can be mentioned a promoter of the glutathione S-transferase II system genes, hsp80 promoter, the promoter of ribulose-2-phosphate carboxylase small subunit gene, and the like. Furthermore, preferred expression vectors may contain, in addition to the above, an enhancer, a splicing signal, polyA addition signal, a selectable marker such as dihydrofolate reductase gene (methotrexate-resistant) and neo gene (G418-resistant), and the like. When an enhancer is contained, the enhancer of SV40, for example, may be inserted into upstream or downstream of the gene.

The transformation of the host with an expression vector may be conducted according to a conventional method well known to a person skilled in the art, which is described in, for example, Current Protocols in Molecular Biology, John Wiley & Sons, 1995. The culturing of transformants can also be conducted according to a conventional method. Purification of a protein from the culture may be conducted by, for example, gel filtration, various column chromatography such as one that employs Sepharose, and the like. When it is expressed as a fusion protein with GST or polyhistidine in the host, it can be easily purified by suitable affinity chromatography.

Given the current state of the art, the promotion of

differentiation such as adventitious shoot formation or branching formation in plants such as roses for which plant regeneration is difficult even by artificial control such as the use of plant hormones can be attained by ligating cDNA or genomic DNA under the control of a constitutive or an inducible promoter, introducing the gene into a plant in a system that employs Agrobacterium, particle gun, or electroporation, and driving the expression thereof.

Furthermore, it will be possible to change the morphology of garden plants, for example changing a standard type into a spray type, by regulating the expression of the gene of the present invention and, as a result, to increase the number of flowers and leaves.

Examples

The present invention will now be explained in further details with reference to the following Examples. Molecular biological procedures were conducted according to Molecular Cloning (Sambrook et al., 1989) unless otherwise specified.

Example 1. Screening of a cytokinin responsive mutant

In order to obtain a mutant that exhibits cytokinin response even in the absence of cytokinin by increasing the amount of the transcribed gene involved in differentiation such as adventitious shoot formation and branching formation, activation tagging was conducted using *Arabidopsis thaliana*.

About 50,000 calluses of *Arabidopsis thaliana* were transformed with a vector pPCVICEn4HPT (Hayashi et al., Science, 258: 1350-1353, 1992) for activation tagging according to the method of Akama et al. (Akama et al., Plant Cell Rep., 12: 7, 1992). Since pPCVICEn4HPT has a strong enhancer sequence derived from the CaMV35S promoter, the transcription of gene adjacent to the enhancer sequence is activated after its insertion into the plant genome. After transformation, the transformed

calluses were cultured in a cytokinin-free medium.

Although the cellular growth of the wild type (non-transformed) *Arabidopsis thaliana* callus is suppressed and adventitious shoots cannot be formed in the cytokinin-free medium, some of the transformed calluses formed adventitious shoots even in the absence of cytokinin. Of them, a transformant that has a high ability of forming adventitious shoots and that forms many adventitious shoots was designated the msh (many shoot) transformant. When seeds obtained from the msh mutant were sown on a conventional agar medium for culturing *Arabidopsis thaliana*, many adventitious shoots were observed on the cotyledons.

Example 2. Isolation of the causative gene MSH of the msh mutant

Genomic DNA was extracted from the msh mutant obtained in Example 1. After this genomic DNA was treated with a restriction enzyme SacI, DNA was purified, and the DNA fragment was circularized with T4 ligase. This was introduced into *Escherichia coli* and then plasmid was collected from *Escherichia coli* that acquired ampicillin resistance. The plasmid thus collected contains genomic sequences adjacent to the right border (RB) of T-DNA and most regions of T-DNA in the msh mutant.

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The nucleotide sequence of 5610 bp genomic DNA adjacent to the RB was determined, and the obtained nucleotide sequence was analyzed by the GENSCAN algorithm (http://CCR-081.mit.edu/GENSCAN.html) to predict the presence of a gene. As a result, it was found that the transcription of the gene which is closest to RB is initiated from a nucleotide at position 882 from RB, and the gene was designated MSH.

Example 3. Isolation of MSH cDNA

From the whole plant of the msh mutant and the wild type *Arabidopsis thaliana*, RNA was extracted, from which mRNA was purified using oligotex dT30 (Nippon Roche).

Using this as a template, a cDNA library was created using the lambda ZAPII cDNA library synthesis kit (Stratagene) according to a method recommended by Stratagene. cDNA libraries of these msh transformant and the wild type *Arabidopsis thaliana* were screened using the MSH gene obtained in Example 2 as a probe. Even screening of about 300,000 clones from the cDNA library derived from the wild type did not yield cDNA corresponding to the MSH gene, suggesting that the expression of the MSH gene in the wild type *Arabidopsis thaliana* is very weak or it is expressed in specific cell alone.

On the other hand, by screening about 20,000 clones of the cDNA library derived from the msh transformant, 31 positive clones were obtained, of which a clone designated M6 was used for the subsequent analysis. The nucleotide sequence of the M6 clone was determined, and the sequence is shown in SEQ ID NO: 1 of the sequence listing. The amino acid sequence corresponding to the nucleotide sequence is shown in SEQ ID NO: 2.

The full-length sequence of this coding region is contained in the MSH gene, and it was revealed that M6 was cDNA corresponding to the MSH gene. From the analysis of the nucleotide sequence of the cDNA, it was found that a protein encoded by the MSH gene has a significant homology with the homeodomain protein, and that the sequence corresponding to the third α helix in the homeodomain conserved between homeodomain proteins is most conserved in the protein encoded by the MSH gene. Furthermore, the amino acid sequence of the protein encoded by the MSH gene had the highest homology with the sequence of WUSCHEL among the homeodomain proteins.

However, even when compared to WUSCHEL having the highest homology with MSH among those reported proteins, the ratio of identical amino acids in the homeodomain is 42% and about 20% in the overall protein, so that it cannot be concluded whether it has a similar function to

WUSCHEL based on the sequence. Of the homeodomain proteins, next to WUSCHEL the proteins of the KN1 type had the second highest homology with MSH protein. When the proteins were compared to MSH within the homeodomain for the homology, the ratio of identical amino acids was 20% or less. During the course of cloning, a cDNA clone of a sequence that had an identity of 86% within the homeodomain and 40% in the entire region with MSH cDNA was isolated and was designated M8. The nucleotide sequence is shown in SEQ ID NO: 3 of the sequence listing, and the corresponding amino acid sequence is shown in SEQ ID NO: 4.

Example 4. Formation of adventitious shoots by overexpression of MSH cDNA

As was predicted in Example 2, it was analyzed whether the overexpression of the MSH gene causes the formation of adventitious shoots. From a binary vector pBE2113GUS (Plant Cell Physiology, 37: 49-59, 1996, obtained from NIAR) the GUS gene was removed by treating with restriction enzymes BamHI/SacI. Instead, a coding region of MSHM6 cDNA was amplified by PCR using primer #170 (5'-GAAGATCTCATCATGTCCTCCTCAAAC-3') (SEQ ID NO: 5) and primer #172 (5'-CGGAGCTCTAAATAAGATAATAGATTGCGC-3') (SEQ ID NO: 6), and then a DNA fragment treated with restriction enzymes BglII/SacI was integrated. By this procedure, the MSH cDNA inserted into the binary vector is placed under the control of an artificial promoter derived from CaMV35S promoter. The plasmid was designated pBE2113MSH.

pBE2113GUS and pBE2113MSH were introduced into the wild type *Arabidopsis thaliana* callus via *Agrobacterium*. Transformed cells were selected using kanamycin-resistance as an index. The transformed callus into which pBE2113GUS had been introduced required cytokinin at the time of adventitious shoot formation, whereas the callus transformed with pBE2113MSH was capable of regenerating adventitious shoots regardless of the

presence of cytokinin. In the presence of cytokinin, calluses transformed with either plasmid regenerated adventitious shoots, whereas the callus transformed with pBE2113MSH regenerated adventitious shoots more rapidly than the callus transformed with pBE2113GUS. Furthermore, *Arabidopsis thaliana* callus that overexpresses CKI1 cDNA, a previously reported sensor histidine of the two-component system can form adventitious shoots in the absence of cytokinin, but the number of adventitious shoots formed was greater in the callus that is overexpressing MSH cDNA.

On the other hand, pBE2113MSH was introduced into germ cells of *Arabidopsis thaliana* using the *Agrobacterium*-mediated vacuum infiltration method (Bechtold et al., C.R. Acad. Sci. Paris, Life Sciences, 316: 1194-1199, 1993; Takashi Araki, Shokubutu Saibo Kogaku Series 4 (Plant Cell Engineering Series 4), Experimental Protocol for Model Plants, pp. 109-113, 1996), and young plants into which the gene was introduced were selected using kanamycin resistance as an index. The transformant *Arabidopsis thaliana* thus obtained was found to have more branching than the wild type strain.

Furthermore, concerning the function of the protein encoded by M8 cDNA that encodes a protein homologous to MSH cDNA obtained in Example 3, a fusion protein of the protein encoded by M8 cDNA and GUS was analyzed by allowing its overexpression in *Arabidopsis thaliana* plant. The coding region of M8 cDNA was amplified by PCR using primer #224 (5'-GCTCTAGAACAATGGCTTCTTCGAATAGACAC-3') (SEQ ID NO: 7) and primer #225 (5'-TCCCCCGGGCTGATCAGATAGTACGAGGCTCC-3') (SEQ ID NO: 8), and then treated with restriction enzymes XbaI/SmaI, and the resulting gene fragment was inserted in between the XbaI/SmaI recognition sites of pBE2113GUS.

The binary vector pBE2113M8GUS thus obtained was introduced into germ cells of *Arabidopsis thaliana* using

the *Agrobacterium*-mediated vacuum infiltration method, and young plants into which the gene had been introduced were selected using kanamycin resistance as an index. The *Arabidopsis thaliana* transformant thus obtained was found to have more branching as does the above Arabidopsis thaliana mutant that overexpressed the above MSHM6 cDNA.

Industrial Applicability

The above data suggested that the gene MSH obtained by activation tagging from *Arabidopsis thaliana* is a homeobox gene involved in adventitious shoot formation, and is estimated to encode a transcription factor of the gene involved in adventitious shoot formation. Based on the results of overexpression of MSH cDNA under the control of the 35S promoter, it was suggested that MSH promotes adventitious shoot formation regardless of the presence of cytokinin, and, besides, that it is also involved in branching of plants.

From the foregoing, it is now possible to control adventitious shoot formation and branching formation from plants or plant cells by regulating the expression of the MSH gene.